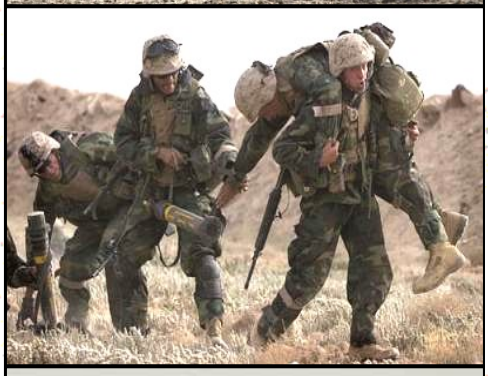

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Michael P. Broderick
M. Steven Oberste
Deborah Moore
Sandra Romero-Steiner
Christian J. Hansen
Dennis J. Faix

Naval Health Research Center

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Naval Health Research Center
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Effect of multiple, simultaneous vaccines on polio seroresponse and associated health outcomes

Michael P. Broderick[a,*], M. Steven Oberste[b], Deborah Moore[b], Sandra Romero-Steiner[b,1], Christian J. Hansen[a], Dennis J. Faix[a]

[a] Operational Infectious Diseases Department, Naval Health Research Center, McClelland & Patterson Roads, Gate 4 / Bldg 315, San Diego, CA 92106 USA
[b] PPLB/DVD/NCIRD Centers for Disease Control and Prevention, 1600 Clifton Road NE, Mailstop G-17, Atlanta, GA 30333 USA

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ABSTRACT

Background: Administration of multiple simultaneous vaccines to infants, children, and military recruits is not uncommon. However, little research exists to examine associated serological and health effects, especially in adults.
Method: We retrospectively examined 416 paired serum specimens from U.S. military subjects who had received the inactivated polio vaccine (IPV) alone or in combination with either 1 other vaccine (<3 group) or 4 other vaccines (>4 group). Each of the 2 groups was subdivided into 2 subgroups in which Tdap was present or absent.
Results: The >4 group was associated with a higher proportion of polio seroconversions than the <3 group (95% vs. 58%, respectively, $p < 0.01$). Analysis of the <3 subgroup that excluded Tdap vs. the >4 subgroup that excluded Tdap showed no difference between them ($p > 0.1$). However, the >4 subgroup that included Tdap had significantly more seroconversions than either the <3 subgroup that excluded Tdap or the >4 subgroup that excluded Tdap ($p < 0.01$). Overall, at least 98% of subjects were at or above the putative level of seroprotection both pre- and post-vaccination, yet at least 81% of subjects seroconverted. In an analysis of 400 of the subjects in which clinic in- and outpatient encounters were counted over the course of 1 year following vaccinations, there was no significant difference between the 2 groups ($p > 0.1$).
Conclusion: A combination of >4 vaccines including IPV appeared to have an immunopotentiation effect on polio seroconversion, and Tdap in particular was a strong candidate for an important role. The dose of IPV we studied in our subjects, who already had a high level of seroprotection, acted as a booster. In addition, there appear to be no negative health consequences from receiving few versus more multiple simultaneous vaccinations.

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1. Introduction

Recruits at all military basic training centers routinely receive three or more vaccinations within several days of arrival at the camp. Multiple simultaneous vaccinations are also very common in immunization schedules for children. Although practical in terms of time, cost, and compliance, the administration of multiple vaccinations raises questions regarding the immediate effect on the immune system, short- and long-term health concerns, and the effect of several simultaneous vaccines on the serological responses of the individual vaccines.

Strategies for study of multiple vaccinations include manipulation of timing of administration and number of vaccines given simultaneously. Pierce and Miller [1] studied Navy basic trainees, one group of which received the regular schedule of 6 vaccines in the first weeks of training and one which received only polio and influenza vaccines in the first 3 weeks and the others during weeks 5 through 9. The latter had significantly lower (approximately 20%) rates of febrile respiratory illness over the entire 10 weeks of training than the former. In this case, the number of vaccines given over the course of 9 weeks was the same but the timing affected the health outcomes. Timing was also investigated by Hotopf et al. [2], who found that receipt of multiple vaccines before deployment was associated with only 1 of the 6 adverse health outcomes that were monitored, while 5 of the 6 adverse

* Corresponding author. Tel.: +1 619 553 8515.
E-mail address: michael.broderick@med.navy.mil (M.P. Broderick).
[1] Current address: Office of Public Health Preparedness and Response, CDC, Atlanta, GA 30333, USA.

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outcomes were associated with receipt of the same vaccinations during deployment.

Investigating administration of multiple vaccinations with respect to the number and type of vaccines, various studies have found either diminished antibody responses [3–5], or poorer health outcomes [6] associated with various combinations of vaccines. A hypothesis for the latter of these effects in war veterans was proposed by Rook and Zumla [7], that a combination of multiple-vaccine schedules, stresses of war, and environmental exposures induced an exaggerated humoral immune response because the vaccines that had been used tend to drive a type 2 helper T cell (Th2) response, which, they argued, could contribute to chronic fatigue, mood change, and allergy. The hypothesis of the association of multiple vaccinations to Gulf War health concerns has been more recently cast in doubt [8–10], and a pediatric study showed no association of multiple vaccinations with increased illness [11].

Many studies have examined the tolerability and immune responses in children to various vaccine combinations, such as the five-component pertussis combination vaccine CPDT-IPV//PRP-T [12], or similar combinations with *Haemophilus influenzae* vaccines. Such combinations have been determined to be immunogenic and safe. A study of children receiving the recommended schedule of vaccines showed no adverse effects on neuropsychological outcomes. [13] Nevertheless, there is evidence of changes in immune response to particular vaccines when given along with others. Lower antibody titers were observed to type 3 polio virus when CPDT-IPV//PRP-T was given in the combined format [12]. Similarly, it was found, for example, that anti-polyribosylribitol phosphate (PRP) response was lower in children when DTaP-IPV-Hib (diphtheria-tetanus-acellular pertussis–inactivated polio vaccine –*H. influenzae* type b vaccine) was given with a conjugate meningococcal vaccine. [14] Likewise, PRP response was diminished when IPV rather than oral polio virus (OPV) was administered with DTaP/Hib [15].

Here we examine the effects of multiple simultaneous vaccinations on IPV-induced seroconversion and seroprotection and subsequent health outcomes in adults. We studied serological responses of individuals who received various combinations of vaccinations, all of which included IPV, and counts of a broad range of clinic encounters as defined by the *International Classification of Diseases, 9th Revision* (ICD-9). Because, as noted above, a form of tetanus/diphtheria/acellular pertussis (Tdap) has been included in many studies of multiple vaccines, we highlighted the inclusion of Tdap among the vaccine combinations.

2. Materials and methods

2.1. Ethics

The Naval Health Research Center Institutional Review Board approved the study protocol (NHRC.2011.0015), and the work was carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki)* for experiments involving humans. The approved study protocol was also reviewed by the Centers for Disease Control and Prevention (CDC) human subjects office and was determined exempt. The subjects were pre-existing de-identified active-duty US military personnel who had provided serum at some time before entering training at a US military recruit center, had been vaccinated soon after arrival at the training center, and had provided serum after vaccination(s) in the time frames noted below, and whose relevant data had been stored the Defense Medical Epidemiological Database and made available by the Armed Forces Health Surveillance Center to the authors.

2.2. Sera

Pre-existing serum pairs were provided by the Department of Defense Serum Repository, stripped of all personally identifying information, and assigned a random identification number so that laboratory personnel were blinded to any study-related information. The Serum Repository regularly collects serum samples from all military personnel as part of its routine surveillance activities and stores them at −30 °C. The serum samples of 0.5 cc each were sent by overnight delivery from the Serum Repository to the Polio and Picornavirus Laboratory Branch, CDC, Atlanta, Georgia, for determination of neutralizing antibody titers.

2.3. Study design

Five study groups were planned, each including IPV. Group 1 was comprised of IPV alone, Group 2 of IPV and Tdap vaccine and any other single vaccine, Group 3 of IPV and any other 1 or 2 vaccines, Group 4 of IPV and 4 other vaccines including Tdap, and Group 5 of IPV and 4 other vaccines not including Tdap. Groups 1, 2, and 3 together were designated as the <3 group and Groups 4 and 5 together as the >4 group.

The subjects must have had 1 serum sample drawn before their vaccination(s) and 1 drawn after at one of three time points: 1–2 months, 3–5 months, or 6–12 months. The pre-vaccination serum sample must have been obtained less than 210 days before the vaccination(s). Data associated with each paired serum set included age, sex, race/ethnicity, military branch, and military grade, as well as outpatient and inpatient encounters in 14 ICD-9 code ranges occurring up to 1 year from the vaccination(s). The outpatient and inpatient encounters recorded under any one of these ICD-9 code ranges were summed for each subject over the course of 1 year from the vaccinations.

There were 416 subjects included in the serology analysis and 400 identified with data on outpatient and inpatient encounters (mean age 21 years, SD = 3.8).

2.4. Polio virus serotypes

Neutralizing antibody titers were determined for polio viruses types 1, 2, and 3, using the 3 Sabin vaccine strains. Stock viral cultures were maintained by passage in HEp-2C cells and frozen at −70 °C in small aliquots [16]. Laboratory personnel were blinded from any study group or patient identifier information.

2.5. Viral particle neutralization assay

Between 80 and 100 median cell culture infectious dose ($CCID_{50}$) of each poliovirus serotype and two-fold serial dilutions of serum (1:8 to 1:1024) were combined and preincubated at 37 °C for 3 h before the addition of HEp-2C cells. After incubation for 5 days at 37 °C and 5% CO_2, plates were stained with crystal violet, and cell viability was measured by optical density in a spectrophotometer. Each specimen was run in triplicate, with parallel specimens from one study subject tested in the same assay run, and the neutralization titers estimated by the Spearman–Karber method [17]. Each run contained multiple replicates of a standard antiserum starting at a 1:32 dilution to assess performance variation. The only serum titers examined were those of polio, not of other vaccines given simultaneously. A titer above the lower cutoff (1:8) was considered seroprotective, [18] a titer below 1:8 was considered negative, and the titer 1:1448 was the upper limit of the assay. Seroconversion was considered to be either a 4-fold rise in titer or a rise from any titer, other than 1:1448, to 1:1448.

2.6. Statistical analysis

Median and geometric mean titers were calculated for groups of vaccines. Logistic regression was used to test the effect of number and grouping of vaccines on seroconversions. Time from vaccination to the subject's subsequent blood draw, sex, race, and age were examined as covariates, with age categorized into 3 ranges: 17–20, 21–26, and ≥27 years. The sums of each subject's inpatient and outpatient encounters were used as the observations in analysis of variance (ANOVA) that tested receipt of <3 vaccines against >4 vaccines. Along with number of vaccines received, sex and race were examined as fixed factors, and continuous age as a covariate. A separate ANOVA was done to test effect of time frame (30-60 days, 61-120 days, 121-180 days, 181-240 days, 241-300 days, and 301-360 days) on outpatient encounters. A multivariate analysis of variance (MANOVA) was done to examine the difference between the counts of in- and outpatient encounters occurring within the ranges of ICD-9 codes. All statistical analyses were performed using SPSS software (IBM, Armonk, NY).

3. Results

The demographics of the subjects by vaccine groupings are shown in Table 1. Subjects were mostly white (61%) and male (86%). For the serology analysis, 57 subjects received IPV alone and 84 received IPV plus 1 other vaccine (4 vaccines accounted for 99% of these). The other 275 subjects received IPV plus 4 vaccines. There were no significant demographic differences between the <3 vaccines and >4 vaccines groups. Vaccine combinations included 16 vaccines (Table 2). Of the subjects with exactly 2 vaccines, the second vaccine was the conjugate meningococcal in 65% of subjects, Td in 17%, measles, mumps, and rubella in 7%, Tdap in 6%, and meningococcal polysaccharide in 5%. In the >4 group combinations, these same vaccines were present in, respectively, 65%, 49%, 20%, 49%, and 30% of subjects, as were attenuated influenza in 40%, split-virus influenza in 33%, and hepatitis A in 65% of subjects.

3.1. Polio seroresponse

Across the 3 antigenic types (P1, P2, and P3) there was agreement of approximately 85% in a comparison of seroconversion, and 98% in putative seroprotection for each of the 498 subjects.

Table 1
Demographics of subjects within each analysis by the dichotomous vaccine grouping.

Demographics	Subject *n* and % of group			
	Serology analysis		Clinic encounter analysis	
	<3 Vaccine group	>4 Vaccine group	<3 Vaccine group	>4 Vaccine group
Sex				
Male	121 (86)	191 (69)	92 (88)	212 (72)
Female	20 (14)	84 (31)	13 (12)	83 (28)
Race/ethnicity				
White	78 (61)	168 (62)	66 (65)	185 (64)
Black	32 (25)	48 (18)	22 (22)	47 (16)
Hispanic	10 (8)	43 (16)	7 (7)	46 (16)
Asian	8 (6)	10 (4)	6 (6)	11 (4)
Age, years				
17–20	80 (57)	161 (59)	59 (56)	173 (59)
21–26	54 (38)	95 (35)	40 (38)	101 (34)
≥27	7 (5)	19 (7)	6 (6)	21 (7)

Because the results of the analyses of the independent variables across the 3 antigens were very similar, only the analyses of P1 are presented here. The race covariates American Indian, Other, and Race Not Stated were removed from analysis because of few observations.

3.1.1. Serum antibody levels

The calculation of median and mean titers was constrained by the fact that the titer assays had a floor of 1:8 and a ceiling of 1:1448, and we report the median and means with this in mind. The post-vaccination median titers were 1:1448 for both the <3 and the >4 groups. However, post-vaccination geometric mean titers were 1:486 for the <3 group and 1:1190 for the >4 group (Table 3). An ANOVA showed this difference to be significant ($p < 0.001$). The statistical comparison, though strictly unadvisable because of the ceiling on the assay values, is suggestive of a real effect if we assume that there were not many wildly high titers (far >1:1448) in the <3 group being masked by the assay ceiling (Table 4).

3.1.2. Seroconversion

Because of the assay ceiling of 1:1448, we could not strictly assess seroconversion as a four-fold rise in titer for all subjects, so the seroconversion analyses here exclude subjects whose

Table 2
Vaccines studied. The occurrences are very similar for the encounter analysis and not shown.

Vaccine	HL7 code	Serology analysis occurrences		
		<3 Group	>4 Group	Sum
Inactivated polio virus (IPV)	010	141	275	416
Meningococcal (total)				
Meningococcal conjugate A, C, Y, W-135	114	55	179	234
Meningococcal polysaccharide A, C, Y, W-135	032	4	83	87
Tetanus/diphtheria (total)				
Td (adult), adsorbed	009	14	136	150
Tdap	115	5	135	140
Hepatitis (total)				
Hepatitis A vaccine, adult dosage	052	0	179	179
Hepatitis A and hepatitis B	104	0	64	64
Hepatitis A vaccine, pediatric/adolescent dosage, 2 dose	083	0	9	9
Hepatitis B, adult dosage	043	0	5	5
Influenza (total)		0	240	240
Influenza virus, live, attenuated, intranasal (LAIV)	111	0	111	111
Influenza virus – split virus (TIV)	015	0	89	89
Novel influenza – H1N1, 2009	127	0	31	31
Influenza virus, not otherwise specified	088	0	9	9
Measles, mumps, and rubella (MMR)	003	6	57	63
Pneumococcal	033	0	1	1
Typhoid VI capsular polysaccharide	101	0	1	1

HL7, health level 7.

pre-vaccination titer was ≥1:1448. The n after eliminating these subjects was 130 for the <3 group and 257 for the >4 group (Table 3). Polio antibody seroconversion from pre-polio vaccination titer to post vaccination titer occurred in 81% of all subjects whose baseline titers were <1:1448. The distribution of baseline titers between the groups was very similar. In particular, the baseline of ≥1:1448 was found in 8% in the <3 group and 7% in the >4 group.

A logistic regression of the <3 versus the >4 vaccine groups, adjusted for sex, race, age category, and time post vaccination to subsequent blood draw, showed large differences in polio seroconversions between the <3 vaccine group and the >4 group (58% vs. 95%, $p < 0.001$; Table 3). We did these same analyses excluding subjects whose pre-vaccination titers were ≥1:1024 but this had no virtually no effect on the results.

The unadjusted logistic regression of the subgroups consisting of IPV plus any 1 vaccine other than Tdap, >4 including Tdap, and >4 plus any vaccines other than Tdap, was significant, with IPV plus any 1 vaccine other than Tdap having a significant lower proportion of seroconversions than >4 including Tdap (Table 3). The adjusted logistic regression was not significant. The unadjusted logistic regression of the subgroups consisting of >4 plus Tdap and >4 plus any vaccine other than Tdap was significant, with >4 including Tdap having a significant higher proportion of seroconversions. The adjusted regression was not significant (Table 3).

The effects noted above involving <3 vaccines were confounded by the fact that the mean time from pre-vaccination serum to vaccination was significantly greater ($p < 0.01$) in the IPV-only group (91 days) than either the IPV + 1 group (59 days) or the >4 group (66 days). However, there was no significant difference between any of the groups in the pre-vaccination geometric mean titer ($p > 0.1$), and the medians were very similar. For this reason, and because there was no a priori reason for suspecting that a difference in mean pre-vaccination period should affect the variable of interest, we did not include pre-vaccination time as a variable in any of the foregoing analyses. However, to allay concern that the difference in pre-vaccination time is indicative of some lurking variable, we did not include the IPV-only group by itself in any of logistic models.

Another confounder was identified as the mean time from vaccination to post-vaccination serum, which was significantly less ($p < 0.05$) in (1) the IPV-only group than the IPV + 1 group, (2) the <3 group than the >4 group, and (3) the >4 with Tdap subgroup than the >4 without Tdap subgroup. However, in the <3 group vs. >4 group comparison ($p < 0.001$), the adjusted model did include the post-vaccination time frames. In addition, in a separate logistic regression including only the <3 group vs. >4 group with post-vaccination time frames, the <3 group still had a lower proportion of seroconversions than the >4 group ($p < 0.001$).

3.1.3. Seroprotection

Overall, 98% and 99% of subjects were at or above the putative level of seroprotection pre- and post-vaccination, respectively, and 98% and 100% were at this level post vaccination in the <3 and >4 groups, respectively.

3.2. Clinic encounters

Overall, 28 of the subjects (7% of all) accounted for the 55 total inpatient visits, and 371 of the subjects (89% of all) accounted for the total 4308 outpatient visits. Across all subjects there was a median of 6 outpatient visits.

The ANOVA for inpatient encounters, with dichotomous vaccine group (<3 vaccines vs. >4), sex, race as fixed factors, and age as a covariate showed no differences between the levels of the factors (the p for each factor was >0.5) (Table 5). The same analysis substituting outpatient encounters as the independent variable showed no difference in any of the levels of the factors except sex ($p = 0.002$)

Table 3
IPV seroconversions and mean and median titers by vaccine grouping, with logistic regressions of the seroconversions relative to the vaccine groupings. For seroconversion analysis, subjects whose baseline titers were ≥1:1448 were excluded.

Vaccine groupings	n	Mean post-vaccination titer	Median pre-vaccination titer (min, max)	Median post-vaccination titer (min, max)	Seroconversions (%)[a]	Unadjusted			Adjusted[b]		
						p value	OR	CI	p value	OR	CI
IPV alone or plus 1 other vaccine (<3 group) vs. IPV plus 4 other vaccines (>4 group)											
<3	141	1:486[c]	1:144 (<1:8, >1:1448)	1:1448 (<1:8, >1:1448)	58						
>4	275	1:1190	1:114 (<1:8, >1:1448)	>1:1448 (1:18, >1:1448)	95	<0.001	13.76	7.13, 26.56	<0.001	11.48	5.53, 23.82
IPV plus just any one not Tdap vs. IPV plus 4 not Tdap vs. IPV plus 4 with Tdap											
IPV + 1 No Tdap	79	1:1065	1:144 (1:18, >1:1448)	1:1448 (<1:9, >1:1448)	89	.032			.118		
>4 With Tdap	135	1:1225	1:91 (<1:8, >1:1448)	1:1448 (<1:72, >1:1448)	99	.009	16.25	1.99, 132.82	.045	9.14	1.05, 79.68
>4 No Tdap	140	1:1157	1:144 (1:18, >1:1448)	1:1448 (<1:18, >1:1448)	91	.671	1.23	0.47, 3.16	.914	1.06	0.36, 3.16
IPV plus 4 not Tdap vs. IPV plus 4 with Tdap											
>4 With Tdap	135	1:1225	1:91 (<1:8, >1:1448)	1:1448 (<1:72, >1:1448)	99						
>4 No Tdap	140	1:1157	1:144 (1:18, >1:1448)	1:1448 (<1:18, >1:1448)	91	.014	0.08	0.01, 0.59	.059	0.13	0.15, 1.08

CI, confidence interval; IPV, inactivated polio vaccine; OR, odds ratio.

[a] The n for the seroconversion calculations, eliminating subjects with a pre-vaccination titer of ≥1:1448 was 130 for the <3 group and 257 for the >4 group.

[b] The adjusted models include the covariates: period since vaccination, sex, race, and age.

[c] The difference in geometric mean titer between the <3 and >4 groups was significant ($p < 0.001$).

Table 4
In- and outpatient encounters by ICD-9 code range and vaccine group.

ICD-9 codes	Inpatient encounters[a]		Outpatient encounters[a]	
	<3 Group	>4 Group	<3 Group	>4 Group
001–139 Infectious and Parasitic Diseases	1	0	78	178
140–239 Neoplasms	0	0	0	5
240–279 Endocrine, Nutritional and Metabolic Diseases, and Immunity Disorders	0	0	11	101
280–289 Diseases of the Blood and Blood-Forming Organs	0	2	9	21
290–319 Mental Disorders	1	20	176	596
320–389 Diseases of the Nervous System and Sense Organs	0	3	90	240
390–459 Diseases of the Circulatory System	0	1	10	15
460–519 Diseases of the Respiratory System	2	5	160	481
520–579 Diseases of the Digestive System	1	1	51	134
580–629 Diseases of the Genitourinary System	1	1	52	97
630–679 Complications of Pregnancy, Childbirth, and the Puerperium	0	3	2	38
680–709 Diseases of the Skin and Subcutaneous Tissue	0	1	54	176
710–739 Diseases of the Musculoskeletal System and Connective Tissue	1	3	194	694
780–799 Symptoms, Signs, and Ill-Defined Conditions	2	6	145	468
All the above	9	1032	46	3276

[a] Visits per subject is in parentheses.

(Table 5). A separate ANOVA on the two >4 groups as factors (>4 without Tdap vs. >4 with Tdap) with sex, race as fixed factors, and age as a covariate, found no difference between them ($p = 0.359$).

The ANOVA that tested the effect of time frame on outpatient encounters showed a significant difference between time frames ($p < 0.001$), with a Tukey post hoc test finding significantly more encounters in the 0–60 day time frame than in any other time frame (the p for all these comparisons <0.01), and significantly fewer encounters in the 301–360 day time frame than in the 30–60, 61–120, and 121–180 time frames (the p for all these comparisons <0.05). There was no interaction between the <3 vs. >4 vaccines as a factor and time frame ($p > 0.9$).

The MANOVA on the dichotomous vaccine group (<3 vs. >4 vaccines) with respect to the ICD-9 code ranges found no difference in either inpatient or outpatient encounters ($p = 0.242$ and $p = 0.718$, respectively).

4. Discussion

The group that received >4 simultaneous vaccinations had significantly more seroconversions to IPV than the group that received <3 vaccines. There was also a significant difference in post-vaccination geometric mean titers between the two groups, although, as stated in the results, the calculation of the mean titers was limited by the fact that there was a ceiling of 1:1448 in the assay. Since the pre-vaccine median polio serum titer among all subjects was 1:114 (only 3 subjects did not have a detectable titer), the vaccination likely acted as a booster (a seroconversion occurring after a seroprotective titer). Although childhood polio vaccine type for these subjects was not available, their ages suggest that most of the subjects had likely previously received OPV and would be expected to have lifelong immunity (Poliomyelitis, Centers for Disease Control and Prevention, (http://www.cdc.gov/vaccines/pubs/pinkbook/downloads/polio.pdf), while the duration of protection of IPV is not known.

The serological difference between the <3 and >4 groups is consistent with the fact that vaccines can create an immunopotentiator effect. Pertussis, for example, is known to act as an adjuvant for other vaccines [19]. In our case, it is unclear which vaccine alone or in combination was acting here as the immunopotentiator for IPV, although Tdap, with its inclusion of pertussis, would be a good candidate [19,20]. Pertussis toxins during infection can evade innate immune mechanisms such as phagocytosis. However, it has recently been reported that pertussis responses are through Toll-like receptor (TLR) type 4 signaling and the stimulation of Th17 lymphocytes [21]. It is possible that increased activation of cellular components may also enhance the response to polio vaccine. The booster response to a fifth dose of combination vaccine that included DTaP and IPV was evaluated by Aase et al. [22] in healthy teenagers, and although these investigators did not report the effect on polio responses, they documented a significant increase in IgG

Table 5
Clinic encounters across all subjects by groupings of vaccines and demographics.

	n[a]	Outpatient encounters	Outpatient encounters, mean	Outpatient ANOVA p value[b]	Inpatient encounters	Inpatient encounters, mean	Inpatient ANOVA p value[b]
Vaccine grouping							
<3	105	1032	9.8	0.686	9	0.09	0.775
>4	295	3274	11.1		46	0.16	
Demographics							
Male	304	2649	8.7	0.002	39	0.13	0.810
Female	96	1657	17.3		16	0.17	
White	251	2695	10.7	0.515	43	0.17	0.799
Black	69	843	12.2		11	0.16	
Hispanic	53	561	10.6		1	0.02	
Asian	17	91	5.4		0	0.00	
17–20 years	232	2616	11.3	0.852	28	0.12	0.383
21–26 years	141	1449	10.3		22	0.16	
≥27 years	27	241	8.9		5	0.17	

ANOVA, analysis of variance; IPV, inactivated polio vaccine; Td, tetanus/diphtheria vaccine; Tdap, tetanus/diphtheria/acellular pertussis vaccine.

[a] The race covariates American Indian, other, and race not stated were removed from ANOVA because of few observations.

[b] The p values for the <3 vs. >4 group comparison included sex, race, and age as factors.

levels and opsonophagocytosis to pertussis. Thus, the combination does not hinder the responses to pertussis antigens. Furthermore, nonspecific stimulation of anti-polio IgA was reported by Fernandes et al. [23] after vaccination of adults with Td vaccine, which could be in agreement with a polyclonal stimulation of memory B cells through TLR4 and TLR9 [24].

Noteworthy, then, is that the unadjusted regression of the subgroup of IPV plus any 1 vaccine excluding Tdap, the subgroup of >4 vaccines including Tdap, and the subgroup of >4 excluding Tdap showed that the two subgroups without Tdap were significantly different from the subgroup with Tdap, and the two subgroups without Tdap were not significantly different from each other. It is possible that the inclusion of Tdap explains a large part of difference found between the groups of <3 vs. >4 vaccines, suggesting that Tdap plays an immunopotentiator role for polio seroconversion.

However, it should be noted that these differences between the groups were found in the unadjusted regressions and not in the adjusted regressions. In addition, it is important to point out that the effect of individual vaccines in the >4 group could not be directly compared in our study because there are many possible combinations that were not equally distributed between Td and Tdap (for example, polysaccharide meningococcal vaccine occurred in 99% of Tdap combinations but only 32% of Td combinations). This limitation also applies to the comparison of the <3 combinations to the >4 combinations. Hepatitis, influenza, and MMR were abundantly present in the >4 groups, but barely represented in the <3 group. One or some combination of those vaccines may have played a role in the difference between the <3 and >4 groups. For example, 240 of 275 subjects in the >4 group received an influenza vaccination compared to none in the <3 group. If influenza plays an immunostimulatory role, then it could perhaps explain a large part of the variance, but as with the other vaccines we were unable to examine it individually. A literature search yielded no reports of immunopotentiation due to co-administration of influenza vaccines and polio vaccines.

Here we underscore other limitations to the analysis of the serology results, namely, (1) that the IPV only group had significantly more mean days from pre-vaccine serum collection to vaccination than other groups, and (2) that there was a significant difference between groups in terms of their mean time from vaccination to post-vaccination serum. In the case of (1), given that there is no a priori reason for this confounding variable in itself to have an effect on the serology results, and that the pre-vaccine titers were not different between vaccine groups, this parameter was not included as a variable in the adjusted model. We believe it important, nevertheless, to mention it as possibly indicative of an unpredicted lurking variable. In the case of (2), the models used in the analysis of the groups designed to include or exclude Tdap, when adjusted for demographic variables and time from vaccination to post-vaccination serum, were not significant, as noted above.

We note as well that our study differed from vaccine-combination studies in infant populations because it centers on a population that has already been primed and a recall of the immune memory elicited during infancy was expected [25]. In addition, our study was limited to the measurement of neutralizing antibodies to polio and we did not measure cytokine levels or markers of cell-mediated immunity. It is possible that simultaneous multiple vaccinations led to an elevated immune response to polio by increasing the recruitment of T-helper cells or the recall of memory B cells. Since we only measured the antibodies to polio, it is possible that the responses to other vaccinations increased as well. We are currently investigating antibody responses of other simultaneously administered vaccines.

Although our study was not designed to provide insight into the mechanism for immune activation that could explain this finding, the literature suggests that multiple simultaneous vaccinations may produce a Th2 response that acts similarly to auto-immune diseases, cancer, or chemical substances. Other studies have investigated this possibility. As mentioned in the introduction, Rook and Zumla [7] hypothesized that some of the symptoms experienced by Gulf War veterans could be explained as a special case of chronic fatigue syndrome with an immunological etiology, related in part to multiple vaccinations and a resulting exaggerated humoral immune response involving a proliferation of Th2 and their associated cytokines. This appeared to be supported by Hotopf and colleagues [2]. To date, however, the Rook and Zumla hypothesis has yet to be demonstrated, and the connection of Gulf War syndrome is questionable [9,10]. [The laboratory-analysis in the companion article in this journal (Hansen et al.) casts further doubt on the hypothesis.] Hotopf et al.'s [8] follow-up study on Gulf War veterans showed the disappearance of the vaccine–symptom association over time.

Here we have found no evidence for an effect of fewer versus more vaccines on subsequent health outcomes as measured by IC9 codes. Over the first year following the vaccination(s), there was no significant difference in either inpatient or outpatient encounters between the <3 and >4 groups, taking account of several demographic covariates. In general, young, active-duty personnel go through medical processing at multiple points, which accounts for some of their outpatient visits, though these would not be expected to affect differences in visits between the vaccine groups. Inpatient encounters were limited to just 7% of the subjects.

There are many examples in the literature of associations of vaccines and subsequent health complaints, such as the alleged pertussis, tetanus, and diphtheria combination's association with allergy/asthma [26–29]. Regarding the latter, most recent and well-controlled studies have failed to find a link [30–35]. It is most likely that any health-associated differences found in military personnel relative to numbers of multiple vaccinations will be related to differences in stress, not vaccines, as we have shown no differences in the number of vaccines on health when the groups have no apparent differences in levels of stress.

U.S. military recruit training centers minimize the number of vaccines given in the beginning of initial entry training by evaluating available medical records, performing serologic studies prior to vaccinating, and delaying vaccines that are not covering immediate health threats in basic training. The pre-vaccination titers we found in this study suggest that the target population is highly protected before entering the military, and the IPV they receive as military recruits acts as a booster to raise titers even higher. We have found no effect on health of multiple simultaneous vaccinations, confirming their safety in the face of the known impact of epidemic diseases in this at-risk population – including histories of devastating outbreaks of influenza, group A streptococcus, adenovirus, and meningococcal disease. Whether administered individually or with other vaccines, IPV offers excellent protection for the military and any population that may be found in areas of active polio transmission. Further study is suggested on whether other vaccines, especially Tdap, have immunopotentiator effects on IPV, and on other vaccines as well.

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6. AUTHORS
Michael P. Broderick, M. Steven Oberste, Deborah Moore, Sandra Romero-Steiner,Christian J. Hansen, & Dennis J. Faix

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Commanding Officer
Naval Health Research Center
140 Sylvester Rd
San Diego, CA 92106-3521

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8. SPONSORING/MONITORING AGENCY NAMES(S) AND ADDRESS(ES)
Commanding Officer
Naval Medical Research Center
503 Robert Grant Ave
Silver Spring, MD 20910-7500

Chief, Bureau of Medicine and Surgery
7700 Arlington Blvd
Falls Church, VA 22042

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14. ABSTRACT
Background: Administration of multiple simultaneous vaccines to infants, children, and military recruitsis not uncommon. However, little research exists to examine associated serological and health effects, especially in adults.
Method: We retrospectively examined 416 paired serum specimens from U.S. military subjects who had received the inactivated polio vaccine (IPV) alone or in combination with either 1 other vaccine (<3 group)or 4 other vaccines (>4 group). Each of the 2 groups was subdivided into 2 subgroups in which Tdap was present or absent.
Results: The >4 group was associated with a higher proportion of polio seroconversions than the <3 group(95% vs. 58%, respectively, $p < 0.01$). Analysis of the <3 subgroup that excluded Tdap vs. the >4 subgroup that excluded Tdap showed no difference between them ($p > 0.1$). However, the >4 subgroup that included Tdap had significantly more seroconversions than either the <3 subgroup that excluded Tdap or the >4 subgroup that excluded Tdap ($p < 0.01$). Overall, at least 98% of subjects were at or above the putative level of sero protection both pre- and post-vaccination, yet at least 81% of subjects seroconverted. In an analysis of 400 of the subjects in which clinic in- and outpatient encounters were counted over the course of 1 year following vaccinations, there was no significant difference between the 2 groups ($p > 0.1$).Conclusion: A combination of >4 vaccines including IPV appeared to have an immune potentiation effect on polio seroconversion, and Tdap in particular was a strong candidate for an important role. The dose of IPV we studied in our subjects, who already had a high level of seroprotection, acted as a booster. In addition, there appear to be no negative health consequences from receiving few versus more multiple simultaneous vaccinations.

15. SUBJECT TERMS
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